

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application. As the prior amendment was not entered, claims are marked to indicate changes from the last entered response filed on April 17, 2009.

1. (currently amended) A method for producing tissue cells wherein the tissue cells are myocardial cells, the method comprising the steps of:
 - (i) an iris-tissue-extirpating step of extirpating iris tissue from the eyeball of the animal;
 - (ii) an iris-pigmented-epithelial-cell-separating step of separating iris pigmented epithelium from the iris tissue thus extirpated;
 - (iii) dissociating the separated iris pigmented epithelium using a trypsin solution;
 - (iv) obtaining pluripotent stem cells by selectively culturing iris pigment epithelial cells by a floated coagulated mass culturing technique, the iris pigmented epithelial cells separated by the steps (i)-(iii) being isolated from an eyeball of an animal, the floated coagulated mass culturing technique comprising culturing the isolated iris pigmented epithelial cells in a culturing media with rotation, the culturing medium comprising serum free medium, and an N2 supplement, and at least one factor selected from the group consisting of fibroblast growth factor (FGF), leukemia inhibitory factor (LIF), and stem cell factor (SCF); and
 - (v) obtaining myocardial cells from the pluripotent stem cells by differentiating the pluripotent stem cells into myocardial cells by culturing the pluripotent stem cells under differentiation inducing conditions comprising culturing the pluripotent stem cells for one to two months in a culture medium comprising fetal calf serum, avian serum, epidermal growth factor (EGF), and fibroblast growth factor 2 (FGF2).
2. (original) The method according to Claim 1, wherein the animal is a chicken, a mouse, a rat, or a human.

3. (previously presented) The method according to Claim 1, wherein the animal is a postnatal individual animal.

4. (previously presented) The method according to Claim 1, wherein the pluripotent stem cells are Oct-3/4 positive and/ or tridermic differentiatable.

5. (cancelled)

6. (previously presented) The method according to Claim 1, wherein the iris-tissue-extirpating step includes:

an iris-tissue-excising stage of excising only iris tissue from the eyeball of the animal;

an enzyme treatment stage of subjecting the excised iris tissue to enzyme treatment; and

an iris-tissue-restoring stage of restoring, by using a culture medium containing serum, the iris tissue weakened by the enzyme treatment.

7-11. (cancelled)

12. (withdrawn) Tissue cells obtained by the method according to claim 1.

13. (withdrawn) The tissue cells according to claim 13, wherein the tissue cells are ectodermal cells or cells derived from ectoderm, mesodermal cells or cells derived from mesoderm, or endodermal cells or cells derived from endoderm.

14. (withdrawn) The tissue cells according to claim 13, wherein the tissue cells forms tissue forming an intravital organ.

15-16. (cancelled)

17. (previously presented) The method of claim 1, further comprising testing for expression of at least one gene specific for myocardial cells.

18. (previously presented) The method of claim 17, wherein the gene specific for myocardial cells is selected from the group consisting of GATA4, Nkx2.5, cMyBP, and myosin.

19-25. (cancelled)

26. (previously presented) The method according to Claim 6, wherein in the enzyme treatment step, the iris tissue is treated in a dispase solution and then treated in an EDTA solution.

27. (previously presented) The method according to Claim 6, wherein in the iris-restoring step, the iris tissue is treated in a culture medium comprising fetal calf serum.

28. (New) The method of Claim 1, further comprising examining the pluripotent stem cells, after step (iv), for absence or presence of expression of an endodermal marker gene, a mesodermal marker gene, and an ectodermal marker gene.

29. (New) The method of Claim 28, wherein the endodermal marker gene is fetoprotein α , the mesodermal marker gene is myosin or MEF2, and the ectodermal marker is pax 6 or tubulin J.